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6. AUTHOR(S)

Adrian J. Dunn, Ph.D. - Principal Investigator
Artur H. Swiergiel, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Louisiana State University Medical Center
P.O.Box 33932
1501 Kings Highway
Shreveport, LA 71130-3932

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13. ABSTRACT *(Maximum 200 words)*

Infections often result in lethargy, hypophagia and body weight loss, the responses not unlike these observed in stress. These responses are thought to be mediated by endotoxins (lipopolysaccharide, LPS) and/or cytokines, such as interleukin-1 (IL-1). The role of cerebral noradrenergic, serotonergic, histaminergic and cholinergic systems in mediating feeding response to LPS and IL-1 was studied. The experiments confirmed that administration of LPS and IL-1, but not of mIL-6 or tumor necrosis factor (TNFα), profoundly reduced milk and food pellet intake, suggesting that the diminution of feeding may be associated with immune activation in general, and IL-1 in particular. Endotoxins and cytokines are known to affect the cerebral neurotransmission. Changes in feeding were induced by LPS and IL-1 and attempts were made to modify the evoked responses applying: 1) Lesions of noradrenergic system (with 6-OHDA and DSP-4) and noradrenergic antagonists; 2) Serotonergic antagonist; 3) Histaminergic antagonists; 4) Cholinergic - muscarinic antagonists. Noradrenergic lesions and α- or β-adrenergic antagonists did not alter the hypophagic effects caused by LPS and mIL-1β. Nor was the hypophagic response affected by the serotonergic, histaminergic and muscarinic antagonists. The results suggest that neither noradrenergic, nor serotonergic, histaminergic and cholinergic system is critical to the hypophagia induced by LPS and mIL-1β.

14. SUBJECT TERMS

Stress, Anxiety, Serotonin, Norepinephrine, Histamine, Behavior, Feeding, Cytokine, Endotoxin

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EFFECTS OF SEROTONERGIC COMPOUNDS ON STRESS-INDUCED BEHAVIOR IN RATS

Final Technical Report : July 01, 1994 - June 30, 1997

I. Introduction

Because our earlier experiments with fenfluramine and serotonin antagonists did not provide encouraging results*, we decided to study effects of endotoxin (lipopolysaccharide, LPS) and interleukin-1, (IL-1) which are known to reduce food intake, and which we have shown also alter cerebral serotonergic and noradrenergic metabolism. Thus it is possible that there is a relationship between the activation of these cerebral neurotransmitter systems and the hypophagia. Specifically, it can be hypothesized that LPS- and IL-1-induced hypophagia are related to the increased noradrenergic and serotonergic activity. This hypothesis has been tested using a variety of neurotransmitter receptor antagonists for their ability to antagonize LPS- or IL-1-induced hypophagia in mice.

Several behavioral experiments were completed.

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* The graduate student engaged on the project decided to take a leave of absence from graduate school, and did not return to continue the experiments. Needless to say this hampered the research progress. We attempted to recruit a replacement student. The reported experiments were performed using assistance of other research students.

A. The role of cerebral serotonergic and noradrenergic systems in mediating feeding response to LPS and interleukin-1.

The parent grant "Cerebral Neurochemical Mechanisms in Stress and Anxiety" was concerned with the relationship between CRF-containing neurons, and catecholaminergic neurotransmitter systems known to be associated with stress and anxiety, principally the noradrenergic neurons. However, there is extensive evidence that serotonergic systems are also activated during stress, including responses to footshock and restraint as well as viral and bacterial infections and inflammation. The significance and the functions of this activation are not understood, but a role for serotonin in the induction of anxiety and the changes in a variety of behaviors has long been suspected. Anxiolytic properties have been suggested for 5-HT_{1A} agonists, and both 5-HT_2- and 5-HT_3-antagonists. The benzodiazepines (BZD) are widely used to treat anxiety, but they have undesirable side effects, including drowsiness, ataxia, and memory impairment, so that their clinical utility is limited. The central serotonergic system has been considered to play a role in the expression of fear and anxiety in animal models of anxiety.

Both the serotonergic and noradrenergic neuronal systems are involved in the central control of functions such as learning, memory, aggression, anxiety, sexual, ingestive and motor behaviors, and sleep. Close morphological and biochemical relationships form the basis of the functional interactions between the two systems. Therefore, as the parent research proposal was concerned with CRF and NE, it made sense to complement it by studying the role of serotonergic and noradrenergic systems in behavioral paradigms that may be affected by anxiety or fear. Because manipulations of the serotonergic systems appear to affect most reliably different feeding behaviors, we focused our attention on feeding. Infections often result in lethargy, hypophagia and body weight loss. These responses are similar to those observed in stress and thought to be mediated by endotoxin (LPS) and cytokines, such as IL-1. In addition, there is substantial evidence that cerebral serotonergic systems are affected by infections and exogenous LPS and cytokines. Similarly, because cerebral noradrenergic systems have been known to be involved in a multitude of behavioral patterns, and are activated during infections and by LPS and interleukin-1, the effects of lesions of

noradrenergic system and of administration of adrenergic receptor antagonists on LPS- and cytokine-induced changes in feeding behavior were studied.

The effects of LPS and cytokines on palatable food motivated ingestive behavior were assessed by observing intake of sweetened milk in a 30-minute period during the light phase. The effects of LPS and cytokines on 22-hour food pellet consumption were also observed.

B. The role of cerebral histaminergic and cholinergic systems in mediating feeding responses to LPS and interleukin-1.

Studies similar to the presented above but using the histaminergic and cholinergic antagonists were conducted. Central administration of IL-1 increases hypothalamic histamine. Activation of H_1-histaminergic receptors in the ventromedial hypothalamus has been shown to reduce food intake and peripheral histamine has been implicated in mediating feeding behavior in rats. Cholinergic activation (parasympathetic) appears to be crucial in both hepatic glucose metabolism and in the optimization of insulin secretion from the pancreas, factors that may have bearing upon feeding. At the central level, administration of carbachol has been shown to depress feeding and anticholinergic scopolamine has been shown to antagonize serotonin-mediated oral behavior.

II. Experiments and Results

A. The role of cerebral serotonergic and noradrenergic systems in mediating feeding response to LPS and interleukin-1.

Acute intraperitoneal injection of endotoxin (LPS, 1-5 μg/mouse) and mIL-1β (100 ng/mouse), but not of mIL-6 (0.5-1.0 μg/mouse) or TNFα (1.0-2.5 μg/mouse) profoundly reduced milk and food pellet intake, suggesting that the diminution of ingestive behavior may be associated with immune activation in general, and IL-1 in particular. Therefore, in the described experiments, only LPS and IL-1 were used to evoke changes in feeding.

Peripheral administration of the mice with a variety of serotonergic compounds, including the 5-HT_{1A} (WAY100135), 5-HT_2/5-HT_{1C} (ritanserin and ketanserin), 5-HT_2/5-HT_{1D} (metergoline) and 5-HT_3 (tropisetron, ICS205930) antagonists and the 5-HT_1/5-HT_{1A} agonists (ipsapirone and 8-OH-DPAT) did not

affect behavioral responses elicited by LPS and IL-1. The hypophagia induced by the immune factors was not attenuated by administration of the above serotonin receptor antagonists.

Similar results were obtained using several adrenergic antagonists. Treatment of the mice with peripherally administered the nonspecific α-adrenergic antagonist (phentolamine), the α_1-adrenergic antagonist (prazosin), or the β-adrenergic antagonist (S-propranolol), either alone or in combination, did not significantly alter the hypophagic responses to IL-1 or LPS. Treatment of the mice with 6-hydroxydopamine (50 μg/2 μl per each cerebral lateral ventricle) or N-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4, 50 mg/kg, ip) elicited significant decreases in NE content in cortex and hypothalamus. However, these noradrenergic lesions did not attenuate behavioral responses induced by IL-1β or LPS. The results suggest that noradrenergic neurons are not primary mediators of the reduction in ingestive behavior evoked by LPS and IL-1.

B. The role of cerebral histaminergic and cholinergic systems in mediating feeding responses to LPS and interleukin-1.

In the next series of studies, histaminergic antagonists were tested against the reductions in food intake induced by LPS and IL-1. The H_1 antagonist, pyrilamine (10 mg/kg, ip) and the H_2 antagonist, cimetidine (50 mg/kg) did not alter the LPS- or IL-1-induced decrease in feeding.

Similarly, the cholinergic muscarinic antagonist, scopolamine (0.1 mg/kg, ip) did not attenuate LPS or IL-1 evoked decrease in feeding.

III. Conclusions

The results from this work confirm that administration of LPS and IL-1 and, by implication, infection or inflammation, decreases food intake. Furthermore, cerebral noradrenergic, serotonergic, cholinergic and histaminergic systems appear not be involved in the hypophagic responses, at least under the conditions under which we tested.

It is well known that is very easy to depress feeding behavior, quite often a byproduct of evoking a nonspecific physiological response or nonfeeding behavioral pattern, but a final common link has not been identified. A potential candidate would be a CRF system. On the other hand, it is notoriously difficult

to stimulate feeding or reverse or attenuate hypophagic responses. The results suggest that many neurotransmitter pathways, mutually supplanting, mediate depression of behavioral patterns in response to LPS and IL-1.

The simultaneous blockade of several neural pathways may be a more viable approach to delineate neural mechanisms underlying LPS/IL-1-induced hypophagia and changes in other behavioral patterns.

Publications

A draft of a manuscript based on the above research and to be submitted to Pharmacology, Biochemistry and Behavior is enclosed.

Data were also reported at "Cytokines and the Brain", a satellite symposium to the Society for Neuroscience 27th Annual Meeting, New Orleans, 23 - 25 October 1997.
Dunn, A. J. and Swiergiel, A. H. Cytokines and behavior: The role of biogenic amines in hypophagia. Abstracts of the symposium, p. 36.